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Differential Immune Responses to *Borrelia burgdorferi* in European Wild Rodent Species Influence Spirochete Transmission to *Ixodes ricinus* L. (Acari: Ixodidae)

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Immune responses to *Borrelia burgdorferi* and their influence on spirochete transmission to *Ixodes ricinus* were analyzed in the natural European reservoir hosts; i.e., the mouse species *Apodemus flavicollis* (yellow-necked mouse) and *Apodemus sylvaticus* (wood mouse) and the vole species *Clethrionomys glareolus* (bank vole), and, in addition, in the laboratory mouse strain NMRI. Naive and preimmunized rodents were infected either by artificially infected *I. ricinus* larvae or by intradermal injection of spirochetes. Independent of the species, all animals developed antibodies to various spirochetal antigens. However, antibodies to the outer surface proteins A (OspA) and B (OspB) were not found in recipients infected via ticks. Rodents of the genus *Apodemus* and of the NMRI strain showed higher levels of *B. burgdorferi*-specific antibodies than those of the species *C. glareolus*. The rate of spirochete transmission to noninfected ticks correlated with both the quality and quantity of spirochete-specific antibodies generated in the various species: high levels of spirochete-specific immunoglobulins correlated with low transmission rates. Furthermore, lower transmission rates were observed with rodents expressing antibodies to OspA and OspB (i.e., intradermally infected or immunized) than with those lacking these specificities (i.e., infected via ticks). The study provides evidence that transmission of *B. burgdorferi* from natural hosts to ticks is controlled by the specificity and quantity of spirochete-reactive antibodies and suggests that immunity to *B. burgdorferi* in natural reservoir hosts is an important regulatory factor in the horizontal transmission of *B. burgdorferi* in nature.

The causative agent of Lyme borreliosis, *Borrelia burgdorferi*, is maintained in nature by vector-competent tick species and susceptible tick hosts, referred to as reservoir hosts (10, 32, 33). For central Europe, the transmission cycles of *B. burgdorferi* seem to be established predominantly by *Ixodes ricinus* (L.) and small and medium-sized mammals (6, 7, 20, 25, 34). In particular, the European mouse species *Apodemus flavicollis* Melchior, *Apodemus sylvaticus* (L.), and *Apodemus agrarius* Pallas (7, 25, 27, 35) and the vole species *Clethrionomys glareolus* Schreber (63) are competent hosts for *I. ricinus* and *B. burgdorferi*.

Previous studies indicate that "specific infectivity" and "reservoir potential" (mathematical terms for the capacity of a given host population to infect ticks [33]) of rodent populations in nature are variable among individual species and are influenced by the ecological situation of the particular location and the season (6, 7, 20, 25, 34, 35, 63, 64). This implies that the intensity of spirochete transmission in nature is determined by numerous intrinsic and extrinsic factors. Although recent studies with the laboratory mouse model suggest that the immune response influences the horizontal transmission of *B. burgdorferi* (14, 16, 17), a detailed analysis of this aspect in natural reservoir hosts from Europe is still lacking.

Investigations of *B. burgdorferi* infection in mice and hamsters have shown that immune sera from infected animals (11, 52, 57) as well as monoclonal antibodies to the outer surface proteins, such as OspA (31 kDa) and OspB (34 kDa) (12, 14, 26, 61) and other still-undefined molecules of spirochetes (24, 51), confer protection against disease and spirochetemia in immunocompromised recipients after experimental or tick-borne challenge with homologous strains. The data suggest that both complement-dependent and complement-independent antibodies are important for preventing disease (5, 38, 49, 52, 56). When generated during infection with large numbers of spirochetes (17) or induced by immunization with recombinant OspA (14, 16), the polyspecific and monospecific antibodies generated were found to reduce the infectivity of mice to ticks, indicating that these immunoglobulins help eliminate spirochetes from the host and/or the tick.

The aim of the present study was to analyze immune responses to *B. burgdorferi* in the European mouse species *A. flavicollis* (yellow-necked mouse) and *A. sylvaticus* (wood mouse) and in the vole species *C. glareolus* after experimental or tick-borne infection with *B. burgdorferi*. The quality and quantity of the spirochete-specific immune responses were related to the course of infection in the recipients as well as to the infectivity to ticks. In addition, the effect of preimmunization of rodents on the subsequent transmission of spirochetes by ticks was investigated. Finally, natural rodent populations occurring sympatrically in a highly endemic focus of Lyme

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borreliosis were investigated for antibody responses to spirochetes and for their infectivity to ticks.

MATERIALS AND METHODS

Bacteria. A low-passage *B. burgdorferi* clone (S12/14) derived from a tick isolate from the Siebengebirge near Bonn (27) was used throughout this study. Spirochetes were cultured in Barbour-Stoenner-Kelly (BSK) II medium as described previously (50) and cloned by single-colony plating. Analysis of DNA and proteins of *B. burgdorferi* clone S12/14 proved that this clone belongs to *B. burgdorferi* sensu stricto (B31 type) (66). S12/14 was moderately pathogenic (data not shown) as assessed in SCID mice by methods described elsewhere (50). For inactivation, spirochetes were irradiated with UV light (λ = 254 nm) at a dose of 800 mJ/cm^2. Inactivation of bacteria was confirmed by recultivation and by xenodiagnosis in NMRI mice inoculated with 10^8 inactivated cells per animal.

Tick colony. The *I. ricinus* colony was bred in the laboratory in Bonn for several years. Interfeeding stages of ticks were kept in glass tubes over a saturated solution of $MgSO_4$ at 20°C. Host-seeking stages were exposed to the natural photophase, while engorged ticks were kept in the dark. Randomly selected larvae or nymphs derived from single-egg batches were regularly tested for contamination with *B. burgdorferi* (10 to 30% of each group). The results showed that all larvae and nymphs used in this study were free of transovarially transmitted spirochetes.

Experimental infection of ticks. *I. ricinus* larvae were infected with a suspension of viable *B. burgdorferi* (S12/14) by a modified capillary feeding technique as described by Gern and colleagues (18). On average, ticks took up 0.05 μl of medium (10^7 cells per ml), corresponding to 500 spirochetes per tick.

Identification of spirochetes in ticks. *B. burgdorferi* infection of ticks was analyzed by an immunofluorescence antibody test (IFAT). Tick smears were air dried on slides, fixed in ice-cold acetone, and incubated with polyclonal immune sera containing antibodies to OspA and OspB (dilution, 1:50). Immune sera were raised against *B. burgdorferi* (S12/14) by intraperitoneal injection of 10^8 viable spirochetes into NMRI mice. As secondary antibodies, fluorescein isothiocyanate (FITC)-labelled goat anti-mouse immunoglobulin G (IgG) antibodies (Sigma) were used.

Xenodiagnosis. *I. ricinus* larvae were used no earlier than 12 weeks after hatching. Xenodiagnosis was performed by exposing each animal to 40 (*Apodemus* spp.) or 80 (*C. glareolus*) noninfected *I. ricinus* larvae. Rodents were kept individually on trays over water. Engorged ticks were collected twice a day, washed two times in sterile phosphate-buffered saline (PBS), and stored as outlined above. Ticks were examined by IFAT after they moulted to nymphs. The percentage of infected ticks per group of mice was used as a measure of the infectivity of rodents to ticks. Differences between groups were determined by Student's *t* test and by the nonparametric Mann-Whitney U test.

Animals and experimental protocols. *A. flavicollis*, *A. sylvaticus*, and *C. glareolus* had been bred in the laboratory for at least two generations before they were used in our studies. Offspring (F_1, F_2, and F_3) were screened for *B. burgdorferi* by xenodiagnosis and Western blot (immunoblot) analysis, IFAT, or cultivation of spirochetes from ear punch biopsies. *B. burgdorferi*-negative 8- to 14-week-old male and female rodents were used for infection experiments. Each group of animals consisted of *A. flavicollis*, *A. sylvaticus*, and *C. glareolus*, NMRI mice were included as controls in groups 1 to 3. Group 1 rodents (n = 12) were immunized by intraperitoneal inoculation with 10^8 inactivated spirochetes and challenged on day 14 after immunization by intradermal (i.d.) inoculation of 10^6 cells per animal, group 2 rodents (n = 8) were immunized and challenged on day 14 after immunization by exposure to 10 artificially infected *I. ricinus* larvae per animal, group 3 rodents (n = 10) were i.d. infected with 10^6 spirochetes, and group 4 rodents (n = 10) were infected by exposure to 10 artificially infected *I. ricinus* larvae per animal. All animals were bled weekly for 12 weeks and tested for antibodies to *B. burgdorferi* by means of IFAT, enzyme-linked immunosorbent assay (ELISA), and Western blot analysis. In addition, animals were inspected for clinical signs of arthritis in the tibio-tarsal joints. Ear punch biopsies were taken on days 10 and 100 postinfection and stored at −20°C. The presence of spirochetes in animals was assessed by xenodiagnosis 3 and 12 weeks after infection. By day 120 after infection, all animals were killed.

Wild rodents were captured between May and October in 1990 and 1991 in a biotope in which *B. burgdorferi* is highly endemic, the Siebengebirge near Bonn, Germany. Live traps were placed as pairs in a trapping point, forming a grid of four by four points spaced 15 m apart. Trapped rodents were investigated for antibodies (by IFAT and Western blot analysis) and for the presence of *B. burgdorferi*.

Western blot analysis, ELISA, and IFAT. Spirochetal antigen of clone S12/14 or strain ZS7 was electrophoretically separated under reducing conditions (sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis) and subsequently blotted onto nitrocellulose (61). Strips were individually incubated with sera (dilution, 1:50). ELISA was performed with either whole sonicated *B. burgdorferi* antigen or purified recombinant OspA as previously described (61). For IFAT, cultured S12/14 cells were air dried on slides, fixed in ice-cold acetone, and incubated with sera (dilution series, 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:360, and 1:720). As secondary antibodies, FITC-labelled goat anti-mouse IgG antibodies (Sigma) or FITC-labelled goat anti-hamster IgG antibodies (Sigma), respectively, were used. Antibody titers were revealed by end point reading. The degree of fluorescence intensity per serum dilution was scored as follows: ++, intense fluorescence of all spirochetes; +, fluorescence of more than 50% of all spirochetes; +−, fluorescence of approximately 50% of cells; and −, fluorescence of less than 50% of cells.

RESULTS

Quantitative and qualitative analysis of antibody responses to *B. burgdorferi* in rodents. Rodents of the species *A. flavicollis*, *A. sylvaticus*, *C. glareolus*, and *Mus musculus* (NMRI) either were inoculated i.d. with inactivated spirochetes (S12/14) (groups 1, 2) or 10^6 viable organisms (group 3) or were exposed to *I. ricinus* larvae previously infected with S12/14 (group 4). Analysis by IFAT of sera from individual rodents taken 2 to 5 weeks later (before challenge) revealed that members of the genus *Apodemus* and NMRI from groups 1 to 3 consistently generated titers of greater than 1:80 of spirochete-specific IgG antibodies, whereas lower and variable titers were observed in *C. glareolus* (Table 1). Antibody titers of sera from group 4 were low, irrespective of the species (<1:40; Table 1). For all rodents, FITC-labelled anti-hamster IgG gave the same results as seen with FITC-labelled anti-mouse IgG, indicating similar affinities of the two secondary antibodies for *B. burgdorferi*-specific antibodies in the three rodent species investigated (data not shown). Sera from naive rodents of all groups did not react with any *B. burgdorferi* antigen. After experimental inoculation with inactivated or viable spirochetes, all individual rodents of groups 1, 2, and 3 rapidly

TABLE 1. Antibody titers (IFAT) of *A. flavicollis* (AF), *A. sylvaticus* (AS), *C. glareolus* (CG), and NMRI mice i.d. inoculated with 10^8 inactivated (groups 1 and 2) or 10^6 viable (group 3) *B. burgdorferi* S12/14 organisms or after tick bites (group 4) 14 to 21 days after primary inoculation

Group	Animal no. (species)	Fluorescence at antibody titer[a] *of:*					
		1:10	1:20	1:40	1:80	1:160	1:320
1	1 (AF)	++	++	++	+	+−	−
	2 (AF)	++	++	+	+	+−	−
	3 (AF)	++	++	++	+	+	+−
	4 (AF)	++	++	++	++	+	+−
	5 (AS)	++	++	++	+	+	+−
	6 (AS)	++	++	+	+	−	−
	7 (AS)	++	++	++	+	+	+−
	8 (CG)	+	+	+−	−	−	−
	9 (CG)	+	+	+−	−	−	−
	10 (CG)	+−	−	−	−	−	−
	11 (CG)	+	+−	−	−	−	−
	12 (NMRI)	++	++	++	++	++	+
2	13 (AF)	++	++	++	+	+	+−
	14 (AF)	++	++	++	+	+	+−
	15 (AS)	++	++	++	++	+	+−
	16 (CG)	++	++	++	+	+	+−
	17 (CG)	−	−	−	−	−	−
	18 (CG)	+	+−	−	−	−	−
	19 (CG)	+	+	+−	−	−	−
	20 (NMRI)	++	++	++	++	++	+
3	21 (AF)	++	+	+	+	+−	−
	22 (AF)	++	+	+	+	+−	+−
	23 (AF)	++	+	+	+	+−	−
	24 (AS)	++	+	+	+−	−	−
	25 (AS)	++	+	+	+	−	−
	26 (AS)	++	+	+	+	+	+−
	27 (CG)	+	+	+	+−	−	−
	28 (CG)	+	+−	−	−	−	−
	29 (CG)	+	+−	−	−	−	−
	30 (NMRI)	++	+	+	+	+−	+−
4	31 (AF)	−	−	−	−	−	−
	32 (AF)	−	−	−	−	−	−
	33 (AF)	+	+−	−	−	−	−
	34 (AS)	−	−	−	−	−	−
	35 (AS)	−	−	−	−	−	−
	36 (AS)	+−	−	−	−	−	−
	37 (AS)	+	−	−	−	−	−
	38 (CG)	−	−	−	−	−	−
	39 (CG)	+	−	−	−	−	−
	40 (CG)	+	+−	−	−	−	−

[a] Fluorescence intensity was scored as described in Materials and Methods. For groups 1 and 2, antibody titers before challenge infection are shown.

developed IgG antibodies to OspA, OspB, flagellin and, in addition, various other proteins with molecular masses of between 18 and 26 kDa and of 70 kDa, independent of the genus (Fig. 1A and B). In some sera, antibodies to proteins with molecular masses of approximately 39 kDa, 60 to 70 kDa, and 80 to 110 kDa were detected. In contrast, rodents infected by ticks (group 4) developed no antibodies to OspA and OspB but did develop antibodies to proteins of 24, 26, 39 to 41, and 70 kDa (Fig. 1C). The presence and absence of OspA-specific antibodies in sera of rodents from groups 1 to 3 and group 4,

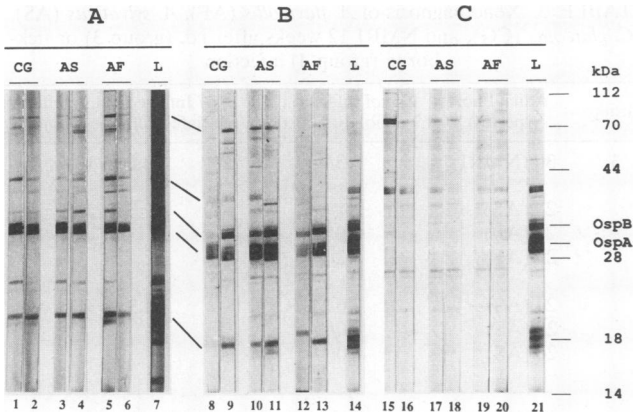

FIG. 1. Western blot analysis of individual sera (dilution, 1:50) from immunized animals (A), i.d. infected animals (B), and rodents infected via ticks (C) on whole-cell lysates of *B. burgdorferi* S12/14. (A) Individual sera from *C. glareolus* (CG, lanes 1 and 2), *A. sylvaticus* (AS, lanes 3 and 4), and *A. flavicollis* (AF, lanes 5 and 6) were taken on day 10 postimmunization with 10^8 irradiated *B. burgdorferi* S12/14 cells in PBS without adjuvant. Lane 7 (L, control) shows the antibody pattern of a laboratory mouse (DBA/2) for strain B31 tested on S12/14. (B) Individual sera from *C. glareolus* (lanes 8 and 9), *A. sylvaticus* (lanes 10 and 11), *A. flavicollis* (lanes 12 and 13), and NMRI (L, lane 14) taken 5 weeks after i.d. infection with 10^6 viable *B. burgdorferi* S12/14 cells. (C) Individual sera from *C. glareolus* (lanes 15 and 16), *A. sylvaticus* (lanes 17 and 18), and *A. flavicollis* (lanes 19 and 20) taken 5 weeks after infection with *B. burgdorferi* S12/14 via 10 artificially infected *I. ricinus* larvae. Lane 21 (L, control) shows the antibody pattern of an NMRI mouse i.d. infected with 10^6 S12/14 cells. For all groups, two representative sera from each rodent species are shown.

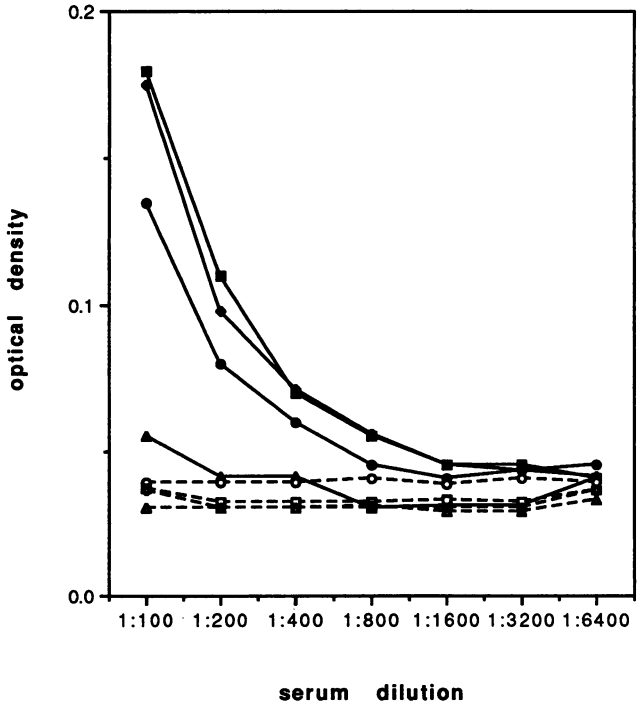

FIG. 2. ELISA with recombinant OspA as antigen. Sera were collected from rodents infected i.d. with 10^6 S12/14 cells or immunized with 10^8 irradiated S12/14 cells (——; rodents 4, 6, 28, and 29) or via 10 artificially infected *I. ricinus* larvae per animal (– – –; rodents 31, 34, 36, and 38). For immunized animals, sera were drawn before challenge. Representative sera are shown. Symbols: ■, rodent 4 (*A. flavicollis*); ◆, rodent 6 (*A. sylvaticus*); ●, rodent 28 (*C. glareolus*); ▲, rodent 29 (*C. glareolus*); ○, rodent 36 (*A. sylvaticus*); □, rodent 31 (*A. flavicollis*); ◇, rodent 34 (*A. sylvaticus*); △, rodent 38 (*C. glareolus*).

respectively, was confirmed by ELISA with purified recombinant OspA (Fig. 2).

Animals previously immunized with inactivated spirochetes (groups 1 and 2) were challenged 14 days postinfection either by i.d. injection of viable spirochetes (group 1) or by exposure to infected tick larvae (group 2). Sera of these animals taken 5 weeks after experimental challenge showed antibody patterns similar to those observed before challenge (Fig. 3A). In contrast, in some immune sera collected 5 weeks after tick-borne challenge (previously specific for OspA and OspB [Fig. 1A]), no antibodies to OspA and OspB were detected by Western blot analysis (Fig. 3B). Antibody titers (IFAT) of sera from preimmunized animals (group 1) taken 5 weeks after i.d. challenge with viable spirochetes were increased by one to two $\log_2$ dilutions compared with titers observed before challenge, whereas the respective titers of antibodies in immunized rodents challenged by tick bites (group 2) progressively declined (one to three $\log_2$ dilutions 5 weeks after rechallenge) (data not shown).

Analysis of spirochete transmission by experimentally and tick-infected naive or preimmunized rodents by xenodiagnosis. Spirochete transmission by all animals was assessed by xenodiagnosis. Rodents previously immunized against *B. burgdorferi* and challenged i.d. (group 1) did not transmit *B. burgdorferi* to *I. ricinus* larvae (0.0%), but two of four immunized animals challenged by ticks (group 2) were infective to ticks (Table 2). For nonimmunized animals, the average rates of transmission to ticks were lower in rodents infected by syringe with 10^6 cells per animal (group 3) (Table 3) than in those infected by *I. ricinus* larvae (group 4) (Table 3). Among members of the genus *Apodemus*, transmission rates differed significantly between groups 3 and 4 (Student's t test, $P < 0.05$; Mann-Whitney U test, $P < 0.05$). In contrast, the infectivity of *C. glareolus* to ticks was similar in groups 3 and 4 and higher than that of *Apodemus* spp. in both groups (44.1% in group 3 and 46.1% in group 4). None of the animals in any of the four groups developed clinical signs of borreliosis, e.g., apparent arthritis in the tibio-tarsal joints.

Occurrence of spirochetes in preinfected ticks after feeding on immunized and naive rodents. Artificially infected *I. ricinus* larvae were allowed to feed on naive animals or on rodents immunized 14 days before (with 10^8 inactivated spirochetes i.d.). Only fully engorged ticks were harvested after natural detachment from rodents caged on trays over water. Fourteen days after repletion, these ticks were tested for the presence of *B. burgdorferi* by means of IFAT. Rates of infection of preinfected ticks fed on immunized rodents with antibody titers of >1:80 and specificities to OspA and OspB were similar (60.0%) to those of ticks fed on rodents with lower titers or on naive animals (58.8%) (Table 4). These data show that no eradication of spirochetes occurred within ticks feeding on immunized rodents under these conditions.

Efficiency of spirochete transmission to ticks in natural rodent populations and prevalence of *B. burgdorferi*-specific antibodies. Rodents (n = 288) were trapped during the summer of 1990 in a defined biotope in which *B. burgdorferi* is highly endemic, near Bonn, Germany. All tick larvae parasitizing these rodents were tested for spirochete infections by IFAT. Those animals which were parasitized by at least one infected tick (approximately one-third of the rodent popula-

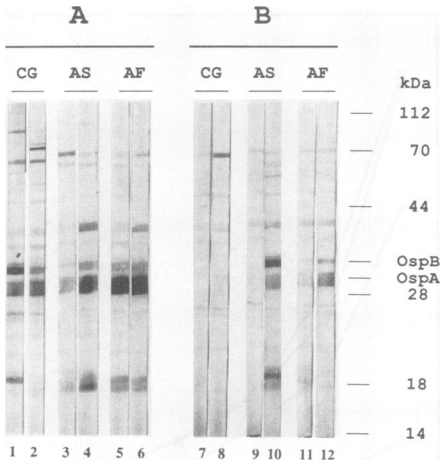

FIG. 3. Western blot analysis of sera (dilution, 1:50) from immunized *C. glareolus* (CG), *A. sylvaticus* (AS), and *A. flavicollis* (AF). Sera were drawn 5 weeks after challenge infection. (A) Animals were challenged with *B. burgdorferi* by i.d. inoculation of 10^6 S12/14 cells per animal. (B) Animals were challenged via 10 experimentally infected *I. ricinus* larvae. Two representative sera from each species are shown. Antibody profiles of sera taken before challenge are shown in Fig. 1A.

tion) were considered to be infective, which was in part confirmed by xenodiagnosis (data not shown). Rates of infection of ticks derived from these animals (50 *A. sylvaticus* and 50 *C. glareolus*) were compared. The frequency of infected ticks derived from *C. glareolus* (282 of 510 [55.3%]) was more than threefold higher than that of infected ticks derived from *A. sylvaticus* (111 of 690 [16.1%]). The frequency of spirochete-infected host-seeking *I. ricinus* larvae from the same area was 1% (2 of 200) as revealed by IFAT.

Fifty randomly taken sera from rodents with unknown states of infectivity trapped in the same biotope in June 1991 were screened for antibodies to *B. burgdorferi* by IFAT and Western blot analysis with strain ZS7 as the target (52, 53). Sera from 20 animals had titers of spirochete-specific antibodies of >1:10 (40%) (data not shown). Western blot analysis revealed that sera from four animals (8%) recognized a protein of 31 kDa and that sera from 12 animals (24%) recognized a protein of 34 kDa (Fig. 4). Eleven sera (22%) strongly reacted with proteins in the molecular mass range of 39 to 41 kDa. Fifteen sera (30%) showed only faint reactions to structures with similar molecular masses. Twenty-five sera reacted with proteins of 70 and 110 kDa. None of the 50 sera recognized proteins in the 18- to 22-kDa range.

TABLE 2. Xenodiagnosis of preimmunized *A. flavicollis* (AF), *A. sylvaticus* (AS), and *C. glareolus* (CG) 12 weeks after experimental (group 1) or tick-borne (group 2) challenge infection

Group	Animal no. (species)	Antibody titer (IFAT)[a]	No. of infected ticks/no. examined
1	1 (AF)	1:80	0/5
	2 (AF)	1:80	0/15
	3 (AF)	1:160	0/10
	4 (AF)	1:160	0/23
	5 (AS)	1:160	0/15
	6 (AS)	1:80	0/11
	7 (AS)	1:160	0/20
	8–11 (CG)	1:40	ND[b]
	12 (NMRI)	1:160	ND
2	13 (AF)	1:160	0/12
	14 (AF)	1:160	2/10
	15 (AS)	1:160	2/13
	16 (CG)	1:160	0/5
	17–19 (CG)	<1:40	ND
	20 (NMRI)	<1:160	ND

[a] Sera were collected before challenge infection. In all cases, antibodies to OspA/B were present.
[b] ND, not determined.

TABLE 3. Xenodiagnosis of *A. flavicollis* (AF), *A. sylvaticus* (AS), *C. glareolus* (CG), and NMRI 12 weeks after i.d. (group 3) or tick-borne (group 4) infection

Group	Animal no. (species)	No. of infected ticks/no. examined	% Infected ticks (total no. infected/no. examined)
3	30 (NMRI)	3/13	23.0 (3/13)
	21 (AF)	1/1	12.5 (3/24)
	22 (AF)	0/4	
	23 (AF)	2/19	
	24 (AS)	6/11	19.3 (6/31)
	25 (AS)	0/2	
	26 (AS)	0/18	
4	31 (AF)	0/11	31.6 (6/19)
	32 (AF)	0/2	
	33 (AF)	6/6	
	34 (AS)	0/9	34.8 (15/43)
	35 (AS)	1/7	
	36 (AS)	13/25	
	37 (AS)	1/2	
3	27 (CG)	6/18	44.1 (15/34)
	28 (CG)	6/10	
	29 (CG)	3/6	
4	38 (CG)	3/6	46.1 (6/13)
	39 (CG)	3/7	
	40 (CG)	ND[a]	

[a] ND, not determined.

DISCUSSION

This study shows that the competent European hosts for *I. ricinus* and *B. burgdorferi*, i.e., the mouse species *A. flavicollis* and *A. sylvaticus* and the vole species *C. glareolus*, develop polyspecific antibody responses after experimental or tick-borne inoculation with *B. burgdorferi*. Antibodies to OspA and OspB were found in rodents only after i.d. inoculation of large numbers of viable spirochetes or after preimmunization with inactivated organisms and not in rodents infected by tick bites. Rodents inoculated by the natural route (i.e., via tick) were more infective to ticks than those which had been needle infected or preimmunized and subsequently challenged. In general, members of the species *C. glareolus* developed lower titers of *B. burgdorferi*-specific antibodies and showed higher transmission rates than members of the genus *Apodemus*.

The finding that outbred European rodents are able to

TABLE 4. *B. burgdorferi* infection in experimentally infected *I. ricinus* after feeding on immunized and naive rodents[a]

Animal no. (species)	Antibody titer	Antibodies to OspA/B	No. of infected ticks/no. examined	% Infected ticks (total no. infected/ no. examined)
32 (AF)	<1:20	−	1/1	58.8 (10/17)
36 (AS)	<1:20	−	2/2	
38 (CG)	<1:20	−	1/3	
39 (CG)	<1:20	−	1/3	
40 (CG)	<1:20	−	2/2	
17 (CG)	<1:20	+	1/2	
19 (CG)	1:20	+	2/4	
13 (AF)	1:160	+	1/4	60.0 (15/25)
14 (AF)	1:160	+	4/7	
15 (AS)	1:160	+	3/4	
16 (CG)	1:160	+	2/3	
AF1 ad	1:320	+	4/5	
AS1 ad	1:160	+	1/3	

[a] The presence or absence of OspA-specific antibodies was revealed by ELISA on recombinant OspA and by Western blot analysis. Abbreviations: AF, *A. flavicollis*; AS, *A. sylvaticus*; CG, *C. glareolus*; AS ad and AF ad, individuals additionally immunized.

develop humoral immune responses to spirochetes after tick-borne infection and/or after experimental inoculation supports studies with the natural reservoir host *Peromyscus leucopus* (white-footed mouse) in the United States (59) as well as studies with laboratory mice (3, 12, 17, 19, 51) and hamsters (45, 58). Studies with the mouse system have shown that production of antibodies to OspA, OspB, and flagellin in response to experimental inoculation with spirochetes is restricted neither by the Igh allotype nor by the H-2 haplotype (55). However, the differential level of antibodies to individual spirochetal antigens in the various rodent species generated during infection, as shown here and before (3, 55), may be controlled by other elements, including a gene(s) not linked to Igh or major histocompatibility complex gene complexes (22).

The lack of OspA- and OspB-specific antibodies in rodents infected by ticks as observed in the present study (Fig. 1C) is also consistent with similar studies with the laboratory models of mice (17, 19), hamsters (45), and dogs (21) as well as with humans (62). The reasons for the differential antibody patterns observed in rodents and dogs after tick-borne or experimental inoculation with spirochetes are not fully understood. However, more recent evidence strongly suggests that the antigenic load is critical for the quality of the antibody response generated. In fact, it was found that only mice inoculated with more than 10^4 spirochetes, but not with fewer or via experimentally infected ticks, developed antibodies to OspA and OspB (51). In view of these findings, we conclude that the number of spirochetes transmitted to rodents by 10 artificially infected tick larvae was below 10^4. In addition (but less likely), the differential antibody pattern may be influenced by factors such as immunomodulatory compounds in tick saliva (44, 65, 67) or antigenic alterations of *B. burgdorferi* during transmission (23, 29, 31, 46, 48, 68).

The data from our experiments show that rodents of the genus *Apodemus* were more infective to xenodiagnostic ticks after tick-borne infection than after i.d. inoculation of large numbers of viable spirochetes (Table 3) or after immunization and subsequent challenge (Table 2). These findings are in accordance with studies on spirochete transmission in laboratory mice (16, 17). The serological data from the present study and previous reports (12, 17, 55) suggest that the infectivity of rodents to ticks is controlled at least in part by antibodies to structures of the outer surface of *B. burgdorferi* (in particular OspA and OspB) either generated during infection or induced by immunization. In fact, previous studies with laboratory rodent models have demonstrated that active immunization with OspA and OspB as well as passive transfer of antibodies with the respective specificities confer protection to recipient mice against syringe or tick-borne challenge with homologous spirochete strains (11–14, 16, 51, 52, 54, 55, 58, 61). This does not, however, exclude the possibility that antibodies with specificities to other spirochetal antigens, such as OspC (15, 68), p39, or additional, yet-undefined molecules (24), also prevent disease or affect transmission. The mechanism(s) by which antibodies control spirochetemia and transmission is only partially understood and has not been addressed in this study. However, the findings that antibodies generated during infection are able to inhibit or kill spirochetes in the presence or absence of complement (4, 56) or even as Fab fragments (47) indicate several means by which the immune system can control infection.

In contrast to that in laboratory mice (17) and in mice of the genus *Apodemus*, the mode of infection in *C. glareolus* (i.e., i.d. infection versus tick-borne) did not influence the infectivity to ticks (groups 3 and 4, Table 3). Most probably this is due to the fact that the titers of spirochete-specific antibodies generated

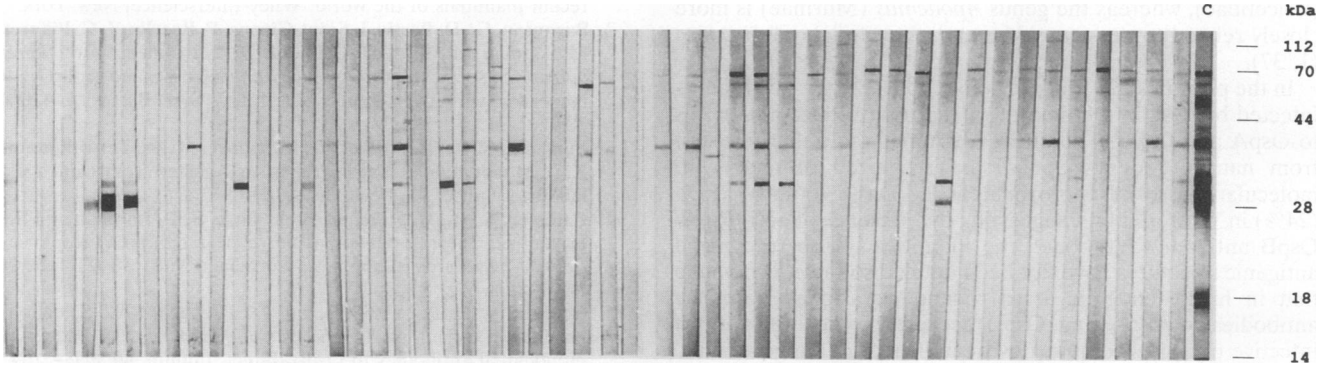

FIG. 4. Western blot analysis of sera (dilution, 1:50) from wild rodents (*C. glareolus*, *A. sylvaticus*, and *A. flavicollis*) trapped during June 1991 in a focus in which *B. burgdorferi* is highly endemic, near Bonn (Germany). Fifty randomly selected sera were tested for *B. burgdorferi*-specific antibodies with strain ZS7 as the target. The positive control (C) is an immune serum from a laboratory mouse (DBA/2) raised against *B. burgdorferi* B31.

in this species were low in most of the individuals of both groups (Table 1).

It has been reported that antibodies induced by OspA not only destroy *B. burgdorferi* in the vertebrate host but may, in addition, eliminate spirochetes within the vector when it is feeding on previously immunized mice (14, 16). Furthermore, partial destruction of spirochetes within ticks has been observed with ticks engorged upon OspE- and OspF-immunized mice (36). This is in contrast to the present data showing that spirochetes are not eliminated in ticks after their feeding on immunized rodent reservoir hosts (Table 4). However, in this study another *B. burgdorferi* strain (S12/14) was used, and rodents were immunized with whole inactivated spirochetes rather than with recombinant outer surface proteins. The data, therefore, indicate that the quality and/or quantity of antibodies generated in response to those spirochetes was sufficient to prevent infection in most of the immunized rodents but not to eliminate spirochetes within ticks. The fact that, in general, naturally infected wild rodents rarely develop titers of *B. burgdorferi*-specific immunoglobulins higher than those observed in the present study makes it rather unlikely that the elimination of spirochetes within ticks feeding on rodents is an event in the natural transmission cycle of *B. burgdorferi*. Determination of whether this assumption is true for any given *B. burgdorferi* sensu lato isolate from the three genomic groups (2, 28, 66) has to await further experimentation.

The finding that the infectivity of *C. glareolus* to ticks is higher than that of members of the genus *Apodemus* and NMRI after both i.d. and tick-borne infection is in line with results of similar studies on *Babesia microti* infection (42, 43). The assumption that *C. glareolus* has a higher degree of reservoir competence for *B. burgdorferi* than *Apodemus* spp. is also corroborated by the present data on natural populations showing that the infectivity to ticks was approximately threefold higher in *C. glareolus* (55.3%) than in *A. sylvaticus* (16.1%) occurring sympatrically in an site of endemicity near Bonn.

In North America, rodents (in particular *P. leucopus*) play a critical role in the transmission cycle of *B. burgdorferi* (10, 32, 59). The level of spirochete infectivity to ticks in *P. leucopus* as well as in hamsters may even exceed that in *C. glareolus* (10, 32, 39). The reasons for this are not known, but it is possible that the infectivity of those reservoir hosts is also related to the quantity and quality of the spirochete-specific immune response. The apparently higher degree of reservoir competence for *B. burgdorferi* of both *C. glareolus* and *P. leucopus* compared with *Apodemus* spp. may reflect phylogenetic relationships among these reservoirs. The genera *Clethrionomys* (Arvivolinae) and *Peromyscus* (Sigmodontinae) are related to hamsters (Cricetidae), whereas the genus *Apodemus* (Murinae) is more closely related to the genus *Mus* and, thus, to laboratory mice (1, 37).

In the present study and previous studies (17, 19, 45) rodents infected by ticks in the laboratory did not produce antibodies to OspA and OspB. Therefore, it is remarkable that few sera from natural rodent populations recognized proteins with molecular masses of approximately 31 kDa (8%) and 34 kDa (24%) in Western blots, suggesting the presence of anti-OspA/OspB antibodies. Although the identities of the recognized antigenic structures have not been defined so far, it is possible that in highly endemic areas rodents occasionally produce antibodies to OspA and OspB because of the high rate of infective tick bites combined with an increased antigenic load. Further studies to analyze the specificity of antibodies in natural rodent populations are under way.

The impact of our findings on the epizootiology of *B. burgdorferi* remains to be elucidated. In many foci of endemicity in Europe, *C. glareolus*, *A. sylvaticus*, and *A. flavicollis* are involved in the transmission cycle of *B. burgdorferi* (7, 25, 34, 35, 63). However, the absolute and relative contributions of each species towards infecting tick populations in individual foci is variable in time and space and appears to be influenced by various intrinsic and extrinsic factors, such as species composition of the vertebrate host cenosis, tick/host attachment ratio, climate, and quality and quantity of the immune response (6, 20, 32, 34, 35, 39, 63, 64). Other possible factors may be related to the heterogeneity among isolates of *B. burgdorferi* sensu lato, a taxon which has recently been subdivided into at least three genospecies (2, 29, 30, 66). So far, the immunogenicities and transmissibilities of strains other than S12/14 in natural hosts from Europe have not been investigated. Moreover, there is emerging evidence that certain reservoir host species (e.g., *C. glareolus*, but not *Apodemus* spp.) acquire resistance to ticks (8, 9, 41–43), a phenomenon which may also affect transmission of *B. burgdorferi* in nature (8, 40, 60). Indeed, recent data from our group demonstrate that the potential of *C. glareolus* populations to infect ticks may be altered by tick densities: a significantly reduced reservoir potential was observed in *C. glareolus* populations from particular foci of *B. burgdorferi* with extremely high tick densities (unpublished observation), suggesting that strong acquired resistance of *C. glareolus* to *I. ricinus* may impair efficient spirochete transmission from vector ticks to this host species.

Altogether, we conclude that naturally acquired immunity in hosts directed towards *B. burgdorferi* and/or the tick vector is an important, density-dependent regulatory factor in the epizootiology of Lyme borreliosis. For a better understanding of the significance of immunity in the transmission cycle of *B. burgdorferi*, further studies on this aspect are required, which should include other tick hosts, in particular reservoir-incompetent ones.

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